इंटरनेट मानक

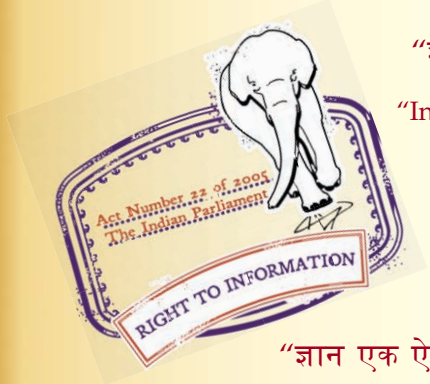
Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

IS 11913 (1986): Tert-Butylhydroquinone (TBHQ) [FAD 8: Food Additives]



"ज्ञान से एक नये भारत का निर्माण"
Satyanarayan Gangaram Pitroda
"Invent a New India Using Knowledge"

"ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है"
Bhartṛhari—Nītiśatakam
"Knowledge is such a treasure which cannot be stolen"

BLANK PAGE

IS : 11913 - 1986
(Reaffirmed 2003)

Indian Standard

SPECIFICATION FOR *Tert*-BUTYLHYDROQUINONE (TBHQ)

UDC 664·099·62 TER

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

Gr 4

July 1987

Indian Standard

SPECIFICATION FOR *Tert*-BUTYLHYDROQUINONE (TBHQ)

Food Additives Sectional Committee, AFDC 19

Chairman	*Representing*
DR T. S. SANTHANAKRISHNAN*	Bush Boake Allen (India) Ltd, Madras
Members	
SHRI B. HANUMANTHA RAU (*Alternate* to DR T. S. Santhanakrishnan)	
DR J. C. ANAND DR S. JINDAL (*Alternate*)	All India Food Preservers' Association, New Delhi
DR S. S. ARYA DR (SMT) T. S. VASUNDHARA (*Alternate*)	Defence Food Research Laboratory, Mysore
SHRIMATI ANITA CHAUDHARY SHRI R. J. VERMA (*Alternate*)	Directorate of Marketing and Inspection (Ministry of Agriculture and Rural Development), New Delhi
SHRI M. V. DESAI DR J. M. TUREL (*Alternate*)	Atul Products Company Limited, Atul
BRIG S. K. DEWAN SHRI K. S. KRISHNAMURTHY (*Alternate*)	Technical Standardization Committee, Army Purchase Organization, New Delhi
SHRI P. K. DHINGRA	Food Research and Standardization Laboratory, Ghaziabad
DRUGS CONTROLLER (INDIA) DEPUTY DRUGS CONTROLLER (INDIA) (*Alternate*)	Ministry of Health and Family Welfare, New Delhi
SHRI R. C. GUPTA SHRI S. N. PANDEY (*Alternate*)	Directorate General of Technical Development, New Delhi
SHRI R. S. IYER DR V. S. MOHAN (*Alternate*)	Glaxo Laboratories (India) Limited, Bombay
SHRI J. C. JAISANI SHRIMATI DEBI MUKHERJEE (*Alternate*)	Directorate General of Health Services (Ministry of Health and Family Welfare), New Delhi
DR E. K. JAYANARAYANAN SHRI D. K. RASTOGI (*Alternate*)	Mohan Meakin Limited, Ghaziabad
JOINT COMMISSIONER (DAIRY DEVELOPMENT) DEPUTY COMMISSIONER (DAIRY DEVELOPMENT) (*Alternate*)	Department of Agriculture (Ministry of Agriculture and Rural Development), New Delhi

*Dr T. S. Santhanakrishnan acted as the chairman at the meeting in which the document was finalized.

(*Continued on page* 2)

(*Continued from page* 1)

Members	*Representing*
SHRI S. KASTHURI PUBLIC ANALYST (*Alternate*)	King Institute, Madras
SHRI J. C. KATHRANI DR S. S. PANDIT (*Alternate*)	Asian Chemical Works, Bombay; *and* All India Saccharin Manufacturers' Association, Bombay
SHRI S. K. KHANNA	Industrial Toxicology Research Centre, Lucknow
SHRI V. K. MENON	Amar Dye Chemi Limited, Kalyan
DR M. J. MULKY DR V. R. SHIRHATTI (*Alternate*)	Hindustan Lever Limited, Bombay
DR A. G. NAIK KURADE	Indian Dehydrated Foods Industries Association, New Delhi
DR B. K. NANDI SHRI M. M. KWATARA (*Alternate*)	Foods and Nutrition Board, Department of Food (Ministry of Food & Civil Supplies), New Delhi
DR C. S. NARAYANAN DR C. ARUMUGHAN (*Alternate*)	Regional Research Laboratory (CSIR), Trivandrum
DR K. M. NARAYANAN DR M. K. JAIN (*Alternate*)	National Dairy Research Institute (ICAR), Karnal
SHRI A. S. RAJADHAYKSHA SHRI S. R. PALEKAR (*Alternate*)	Corn Products Company (India) Pvt Ltd, Bombay
DR R. RAJAGOPALA RAO DR R. SESHADRI (*Alternate*)	Central Food Technological Research Institute (CSIR), Mysore
SHRI N. RAMAMURTHY	Indian Confectionery Manufacturers' Association, New Delhi
REPRESENTATIVE	The Federation of Hotel and Restaurant Association of India, New Delhi
DR A. K. ROY SHRI D. S. CHADHA (*Alternate*)	Council for Indian Food Trade and Industry, New Delhi
SHRI M. W. SHENDE SHRI K. P. RANE (*Alternate*)	Hickson & Dadajee Ltd, Bombay
DR I. S. SHENOLIKAR DR RAMESH BHAT (*Alternate*)	National Institute of Nutrition, Hyderabad
SHRI I. A. SIDDIQI	Directorate of Vanaspati, Vegetable Oils & Fats, Ministry of Food and Civil Supplies, New Delhi
DR T. V. SUBBAIAH DR R. K. MEHTA (*Alternate*)	Indian Chemical Manufacturers' Association, Calcutta
SHRI S. B. SULE SHRI D. B. BEDEKAR (*Alternate*)	Naarden (India) Limited, Bombay
SHRI A. TIWARI DR R. M. OZA (*Alternate*)	Central Salt & Marine Chemicals Research Institute, Bhavnagar
DR G. M. TIWARI DR C. P. S. MENON (*Alternate*)	Britannia Industries Ltd, Bombay
SHRI M. B. TURAKHIA SHRI B. J. NAVLOOR (*Alternate*)	Idachem Industries Pvt Ltd, Bombay
SHRI P. C. VIN	Vin Flavours, Vadodara
SHRI T. PURNANANDAM, Director (Agri & Food)	Director General, BIS (*Ex-officio Member*)

Secretary

SHRIMATI SHASHI SAREEN
Deputy Director (Agri & Food), BIS

(*Continued on page* 14)

Indian Standard

SPECIFICATION FOR *Tert*-BUTYLHYDROQUINONE (TBHQ)

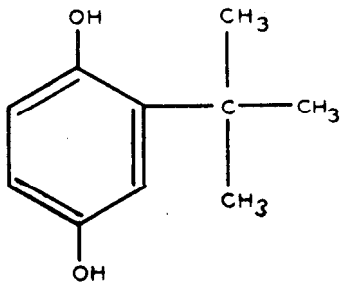
0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 28 November 1986, after the draft finalized by the Food Additives Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 With the increased production of processed foods, manufacturers have started adding a large number of substances, generally in small quantities, to improve the appearance, flavour, texture or storage properties and in some cases to enhance the nutritive value of the processed foods. As certain impurities in these substances have been found to be harmful, it is necessary to have a strict quality control of these food additives. A series of standards is, therefore, being prepared by this Bureau to cover purity and identification of these substances. It is hoped that these standards would help in checking purity which requires to be checked at the stage of manufacture, for it is extremely difficult (and in many cases impossible) to detect the impurity once these substances have been added to the processed foods. Besides, these standards are intended to guide the indigenous manufacturers in making their product conform to specifications that are accepted by scientists, health authorities and international bodies.

0.3 *Tert*-butylhydroquinone (TBHQ) is a white, crystalline solid having a characteristic odour. It is used as an antioxidant in edible oils and fats and whole milk powder in the country.

0.4 *Tert*-butylhydroquinone is also known as mono-*tert*-butylhydroquinone, *tert*-butylquinol, and 2-tertbutyl-1,4-dihydroxybenzene. Its empirical formula is $C_{10}H_{14}O_2$. Its molecular weight is 166·22 and structural formula is:



0.5 In the preparation of this standard considerable assistance has been derived from the Food Chemical Codex, Published National Academy of Sciences and National Research Council, Washington DC, USA.

0.6 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS : 2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

---

1. SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and test for *tert*-butylhydroquinone (TBHQ).

2. REQUIREMENTS

2.1 Description — *Tert*-butylhydroquinone shall be a white crystalline solid having a characteristic odour. It shall be soluble in alcohol and ether but practically insoluble in water.

2.2 Identification — Dissolve a few milligrams of the product in 1 ml of methanol and add a few drops of 25 percent solution of dimethylamine in water. A pink to red colour shall be produced.

2.3 Melting Range — The melting range of the product shall be between 126·5 and 128·5°C.

2.4 The material shall also conform to the requirements given in Table 1.

3. PACKING, STORAGE AND MARKING

3.1 The material shall be filled in well-closed containers, so as to preclude air contamination of the contents with metal or other impurities.

3.2 Storage — The material shall be stored in a cool and dry place.

3.3 Marking — Each container shall be marked legibly and indelibly to give the following information:

a) Name and type of the material including the words 'Food Grade';

b) Name and address of the manufacturer;

c) Minimum net mass,

d) Batch or code number, and

e) Date of manufacture.

TABLE 1 REQUIREMENTS FOR *tert*-BUTYLHYDROQUINONE (TBHQ)

(*Clause* 2.4)

Sl No.	Characteristic	Requirement	Method of Test, Ref to Cl of	
			Appendix A	IS : 1699-1974*
(1)	(2)	(3)	(4)	(5)
i)	Purity as $C_{10}H_{14}O_2$, percent by mass, *Min*	99·0	A-1	—
ii)	*t*-Butyl-*p*-benzoquinone, percent by mass, *Max*	0·2	A-2	—
iii)	2, 5-Di-*t*-butylhydroquinone, percent by mass, *Max*	0·2	A-3	—
iv)	Hydroquinone, percent by mass, *Max*	0·1	A-3	—
v)	Arsenic (as As), mg/kg, *Max*	3	—	9
vi)	Heavy metals (as Pb), mg/kg, *Max*	10	A-4	—
vii)	Toluene, mg/kg, *Max*	25	A-5	—
viii)	Ultraviolet absorbance (polynuclear hydrocarbons)	passes test	A-6	—

*Methods of sampling and test for food colours (*first revision*).

3.3.1 The container may also be marked with the Standard Mark.

NOTE — The use of the Standard Mark is governed by the provisions of the Bureau of Indian Standards Act, 1986 and the Rules and Regulations made thereunder. The Standard Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well defined system of inspection, testing and quality control which is devised and supervised by BIS and operated by the producer. Standard marked products are also continuously checked by BIS for conformity to that standard as a further safeguard. Details of conditions under which a licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

4. SAMPLING

4.1 The representative samples of the material shall be drawn and conformity of the material to the requirements of this specification shall be determined according to the procedure prescribed in **3** of IS : 1699-1974*.

5. TESTS

5.1 Tests shall be carried out by the methods specified in col 4 and 5 of Table 1.

*Methods of sampling and test for food colours (*first revision*).

5.2 Quality of Reagents — Unless specified otherwise pure chemicals and distilled water (*see* IS : 1070-1977*) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

APPENDIX A

(*Table* 1)

METHOD OF TEST FOR *tert*-BUTYLHYDROQUINONE (TBHQ)

A-1. PURITY

A-1.1 Reagents

A-1.1.1 *Methanol*

A-1.1.2 *Sulphuric Acid* — 1 N.

A-1.1.3 *Diphenylamine Indicator* — 3 mg of *p*-diphenylaminesulphonic acid sodium salt per ml of 0·1 N sulphuric acid.

A-1.1.4 *Ceric Sulphate* — 0·1 N.

A-1.2 Procedure — Transfer about 170 mg of the sample, ground to a fine powder and accurately weighed, into a 250-ml wide-mouthed Erlenmeyer flask, and dissolve in 10 ml of methanol. Add 150 ml of water, 1 ml of 1 N sulphuric acid, and 4 drops of diphenylamine indicator. Titrate with 0·1 N ceric sulphate to the just complete colour change from yellow to red-violet. Record the volume, in ml of 0·1 N ceric sulphate required. If HQ and DTBHQ are present in the sample they will be included in the titration and the correction as given in the formula shall be applied.

A-1.3 Calculation

$$\text{Purity, as } C_{10}H_{14}O_2, \text{ percent by mass } (A) = \frac{8{\cdot}311 \times N (V - 0{\cdot}1)}{M}$$

where

V = volume, in ml, of 0·1 N ceric sulphate required;

N = normality of the ceric sulphate solution; and

M = mass, in grams, of the sample taken.

NOTE — 0·1 ml represents the volume of ceric sulphate solution consumed by the primary oxidation products of *tert*-butylhydroquinone ordinarily present in the sample.

*Specification for water for general laboratory use (*second revision*).

A-1.3.1 If HQ and DTBHQ are present in the sample they will be included in the titration and the following correction shall be applied:

Purity, as $C_{10}H_{14}O_2$, corrected percent by mass = A − (percent HQ × 1·51) − (percent DTBHQ × 0·75)

A-2. *t*-BUTYL-*p*-BENZOQUINONE

A-2.1 Apparatus

A-2.1.1 *Spectrophotometer* — A double-beam infrared spectrophotometer with matched 0·4 mm liquid sample cells with calcium flouride windows.

A-2.2 Reagents

A-2.2.1 *Monotertiary-butyl-p-benzoquinone Reference Standard*

A-2.2.2 *Carbon Tetrachloride*

A-2.3 Procedure

A-2.3.1 *Preparation of Standard* — Transfer about 10 mg of accurately weighed monotertiary-butyl-*p*-benzoquinone Reference Standard, into a 10-ml volumetric flask. Dissolve in carbon tetrachloride, dilute to volume with the same solvent and mix.

A-2.3.2 *Preparation of Sample* — Transfer about 1 g of the sample, accurately weighed and previously reduced to a fine powder in a high speed blender, into a 10-ml volumetric flask. Dilute to volume with carbon tetrachloride, and shake for 5 minutes to extract the *t*-butyl-benzoquinone. Filter through a millipore filter or equivalent before use.

A-2.3.3 Fill the reference cell with carbon tetrachloride and the sample cell with the standard preparation (**A-2.3.1**). Place the cells in the respective reference and sample beams of the spectrophotometer and record the infra spectrum from 1 600 to 1 775 cm^{-1}. On the spectrum draw a background line from 1 612 to 1 750 cm^{-1}, and determine the net absorbance (A_s) of the standard preparation at 1 659 cm^{-1}. Similarly, obtain the spectrum of the sample preparation (**A-2.3.2**), and determine its net absorbance (A_u) at 1 659 cm^{-1}.

A-2.4 Calculation

$$t\text{-butyl-}p\text{-benzoquinone, percent by mass} = \frac{A_u}{A_s} \times \frac{W_s}{W_u} \times 100$$

where

A_u = net absorbance of the sample preparation;

A_s = net absorbance of the standard preparation;

W_s = exact mass, in mg, of the Reference Standard taken; and

W_u = exact mass, in mg, of the sample taken.

A-3. 2,5, Di-*t*-BUTYLHYDROQUINONE AND HYDROQUINONE

A-3.1 Apparatus

A-3.1.1 *Gas Chromatograph* — of a suitable type equipped with a thermal conductivity detector, containing a 0·61 m × 6·35 mm (O.D.) stainless steel column packed with 20 percent Silicone SE-30, by mass and 80 percent Diatoport S (60/80-mesh), or equivalent materials.

A-3.1.1.1 *Operating conditions* — The operating parameters may vary depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

a) *Column temperature* — programmed from 100 to 270°C at 15°C per minute;

b) *Injection port temperature* — 300°C;

c) *Carrier gas* — helium or nitrogen flowing at a rate of 100 ml per minute;

d) *Bridge current* — 140 ma; and

e) *Sensitivity* — 1 × for integrator, 2 × for recorder.

A-3.2 Reagents

A-3.2.1 *Hydroquinone, 2,5-di-t-butylhydroquinone and Methyl Benzoate Stock Solution* — Weigh accurately about 50 mg each of hydroquinone (HQ) 2,5-di-*t*-butylhydroquinone (DTBHQ), and methyl benzoate (internal standard). Transfer into separate 50-ml volumetric flasks, dilute to volume with pyridine and mix.

A-3.3 Procedure

A-3.3.1 *Calibration Standard* — Into separate 10-ml volumetric flasks, add 0·50, 1·00, 2·00 and 3·00 ml of the HQ stock solution. Then to each flask add 2·00 ml of methyl benzoate (internal standard) stock solution, dilute each to volume with pyridine and mix. In the same manner prepare four DTBHQ calibrating solutions. Prepare the trimethylsilyl derivative of each solution as follows -- add 9 drops of calibration solution to a 2-ml serum vial, cap the vial, evacuate with a 50-ml gas syringe, add 250 µl of N,O-bis-trimethylsilyl-acetamide, and heat at about 80°C for 10 minutes. Chromatograph 10 µl portions of each standard in duplicate, and plot the concentration ratio of HQ to internal standard (X-axis) against response ratio of HQ to internal standard (Y-axis). Plot the same relationship between DTBHQ and the internal standard.

A-3.3.2 *Sample Preparation and Procedure* — Transfer about 1 g of the sample, accurately weighed, into a 10-ml volumetric flask. Add 2·00 ml of the methyl benzoate internal standard stock solution, dilute to volume with pyridine and mix. Prepare the trimethylsilyl derivative as

described in **A-3.3.1** and then chromatograph duplicate 10-μl portions to obtain the chromatogram. The approximate peak times in minutes are: Methyl benzoate-2·5; trimethylsilyl derivative of HQ-5·5; trimethylsilyl derivative of *tert*-butylhydroquinone-7·3; trimethylsilyl derivative of DTBHQ-8·4.

A-3.4 Calculation

A-3.4.1 Determine the peak area (response) of interest by automatic integration or manual triangulation. Calculate the response ratio of HQ and DTBHQ to internal standard. From the calibration curves determine the concentration ratio of HQ and DTBHQ to internal standard, and calculate percent HQ and percent DTBHQ as follows:

$$\text{HQ or DTBHQ, percent by mass} = Y \times I \times \frac{10}{S}$$

where

Y = concentration ratio (X-axis on calibration curve);

I = percent (w/v) of internal standard in the sample preparation; and

S = mass, in grams, of the sample taken.

A-4. HEAVY METALS

A-4.1 Reagents

A-4.1.1 *Ammonia Solution* — Dilute 400 ml of ammonium hydroxide (28 percent) to 1 000 ml with water.

A-4.1.2 *Hydrochloric Acid* — 10 percent.

A-4.1.3 *Lead Nitrate Stock Solution* — Dissolve 159·8 mg of lead nitrate in 100 ml of water containing 1 ml of nitric acid. Dilute with water to 1 000 ml and mix. Prepare and store the solution in lead-free glass containers.

A-4.1.4 *Standard Lead Solution* — Dilute 10 ml of lead nitrate stock solution, accurately measured, with water to 100 ml. Each ml of the solution so prepared contains the equivalent of 10 μg of lead ion (Pb). Prepare the solution on the day of use.

A-4.1.5 *Nitric Acid* — 10 percent (v/v).

A-4.1.6 *Sulphuric Acid* — 94·5 to 95·5 percent (v/v).

A-4.1.7 *Acetic Acid* — 6 percent (m/v).

A-4.1.8 *Hydrogen Sulphide* — A saturated solution of hydrogen sulphide made by passing H_2S in cold water.

A-4.2 Procedure

A-4.2.1 *Solution A* — Take 2 ml of the standard lead solution in a 50-m Nessler tube and add 23 ml of water. Adjust the *p*H to between 3·0 and 4·0 by addition of acetic acid or ammonia solution. Dilute with water to 40 ml and mix.

A-4.2.2 *Solution B* — Place 500 mg of the sample, accurately weighed in a suitable crucible, add sufficient nitric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulphuric acid and cautiously heat until white fumes are evolved. Then ignite, preferably in a muffle furnace at 500 to 600°C until the carbon is all burnt off. Cool, add 4 ml of dilute hydrochloric acid, cover and digest on a steam bath for 10 to 15 minutes. Uncover and slowly evaporate on a steam-bath to dryness. Moisten the residue with one drop of hydrochloric acid, add 10 ml of hot water and digest for 2 minutes. Add dropwise ammonia solution until the solution is just alkaline to litmus paper, dilute with water to 25 ml and adjust the *p*H to between 3·0 and 4·0 (*p*H indicator paper) by the addition of diluted acetic acid. Filter, if necessary. Wash the crucible and the filter with 10 ml of water. Transfer to a 50-ml Nessler tube. Dilute the combined filtrate and washing with water to 40 ml and mix.

A-4.2.3 To each tube add 10 ml of freshly prepared hydrogen sulphide, mix and allow to stand for 45 minutes and view down over a white surface. The colour of solution *B* shall not be darker than that of Solution *A*.

A-5. TOLUENE

A-5.1 Apparatus

A-5.1.1 *Gas Chromatograph* — of a suitable type equipped with a flame ionization detector containing a 3·66 m × 3·18 mm (O.D) stainless steel column packed with 10 percent Silicone SE-30, by mass, and 90 percent Diatoport S (60/80-mesh), or equivalent material.

A-5.1.1.1 *Operating conditions* — The operating parameter may vary depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

a) *Column temperature* — programmed from 70 to 80°C at 15°C per minute and held.

b) *Injection port temperature* — 275°C.

c) *Carrier gas* — helium or nitrogen flowing at a rate of 50 ml per minute.

d) *Cell temperature* — 300°C.

e) *Hydrogen and air settings* — 138 kPa each.

f) *Sensitivity* — 1×10^2.

A-5.2 Reagents

A-5.2.1 *Toluene*

A-5.2.2 *Octyl Alcohol*

A-5.3 Procedure

A-5.3.1 *Preparation of Standard Toluene Solution* — Prepare a solution of toluene in octyl alcohol containing approximately 50 μg per ml and calculate the exact concentration (C_T) in percent (m/v).

A-5.3.2 *Preparation of Sample Solution* — Transfer about 2 g of the sample accurately weighed, into a 10-ml volumetric flask. Dissolve in octyl alcohol. Dilute to volume with the same solvent and mix. Calculate the exact concentration of the solution (C_S) in percent (m/v).

A-5.3.3 Inject a 5-μl portion of the standard solution into the chromatograph, and measure the height of the toluene peak on the chromatogram. The toluene retention time is 3·3 minutes. Similarly obtain the chromatogram on a 5-μl portion of the sample solution, and measure the height of the toluene peak. Calculate the percentage of toluene in the sample.

A-5.4 Calculation

$$\text{Toluene, mg/kg} = \frac{H_S}{H_T} \times \frac{C_T}{C_S} \times 10^6$$

where

H_S = height of the toluene peak of the sample solution,

H_T = height of the toluene peak of the standard solution,

C_S = concentration in percent (m/v) of the sample solution, and

C_T = concentration in percent (m/v) of the standard solution.

A-6. ULTRAVIOLET ABSORBANCE

A-6.1 Reagents

A-6.1.1 *L-Ascorbic Acid*

A-6.1.2 *Ethanol*

A-6.1.3 *Isooctane*

A-6.1.4 *Anhydrous Sodium Sulphate*

A-6.1.5 *Hexadecane*

A-6.2 Procedure

A-6.2.1 Dissolve 1 g of L-ascorbic acid in 100 ml of ethanol and 100-ml of water contained in a 500-ml separator (S-1). Transfer about 50 g of the sample, accurately weighed into the separator. Shake to dissolve, then add 50-ml of isooctane and extract for 3 minutes. After the phases have separated, drain the lower aqueous phase into a second 500-ml separator (S-2), then after 1 minute of further separating, drain the lower layer into the separator (S-2). Add a second 50-ml portion of isooctane to the aqueous solution in S-2 and repeat the extraction procedure as previously described, drawing off the lower, aqueous layer into a third 500-ml separator (S-3). Add a third 50-ml portion of isooctane to the aqueous solution in S-3 and repeat the extraction procedure as previously described, drawing off and discarding the lower aqueous layer.

A-6.2.2 Extract each isooctane solution (that is, the solution in S-1, S-2, S-3), with two 100-ml portions of a 0·5 percent solution of ascorbic acid in ethanol-water (25 : 75). Shake each mixture for 1 minute, allow the phases to separate, and discard the lower, aqueous layers. Next, extract each isooctane solution with two 100-ml portions of a 5 percent solution of ethanol in water, and discard the lower, aqueous layers. Finally, wash each solution twice with 100-ml of water, and discard the washes.

A-6.2.3 Lightly pack a standard size chromatographic tube with 100 g of anydrous sodium sulphate, and wash the packed column with 75 ml of isooctane, discarding the wash. Filter the isooctane solution from S-1 through the column, and collect filtrate in a 500-ml distillation flask. Wash S-1 with the isooctane solution contained in S-2, and then pour the solution onto the column, collecting the filtrate in the flask. Wash S-2 and S-1, successively, with the isooctane solution in S-3, and filter the solution through the column as before. Wash S-3, S-2, and S-1 in that order and in tandem with two successive 25-ml portions of isooctane, and pass the washings individually through the column and into the flask. Let the column drain completely.

A-6.2.4 Add 2-ml of hexadecane and 2 boiling stones to the 500-ml distillation flask containing the combined isooctane extracts, and attach the flask to a suitable vacuum distillation assembly. Evacuate the assembly to about one-third atmosphere, then immerse the flask in a steam bath, and distill the solvent. When isooctane stops dripping into the receiver, turn off the vacuum, wash down the walls of the flask with 5 ml of isooctane added through the top of the distillation head, then replace the thermometer and again evacuate. The isooctane should distill over in about 1 minute. At the end of this distillation add another 5-ml portion of isooctane and repeat the stripping procedure.

A-6.2.5 Quantitatively wash the residue from the distillation flask into a 50-ml volumetric flask with isooctane, dilute to volume with isooctane, and mix. Determine the ultraviolet absorption spectrum of the solution in a 5-cm silica cell from 400 mμ to 250 mμ, with a suitable spectrophotometer, using isooctane as the blank. Determine the absorbance of a solvent control by following the above procedure in every detail, but with the sample omitted. From the sample spectrum determine the maximum absorbance per cm path length in each of the following wavelength intervals: (a) 280-289 mμ; (b) 290-299 mμ; (c) 300-359 mμ; and (d) 360-400 mμ. Calculate the maximum net absorbance per cm in each interval by subtracting from the sample absorbance the corresponding absorbance per cm of the solvent control. The following net absorbance values are not exceeded at the indicated intervals: (a) 0·15; (b) 0·12; (c) 0·08; and (d) 0·02.

(*Continued from page* 2)

Preservatives, Antioxidants, Emulsifying and Stabilizing Agents Subcommittee, AFDC 19 : 3

Convener	*Representing*
DR I. A. SIDDIQI	Directorate of Vanaspati, Vegetable Oils and Fats (Ministry of Food and Civil Supplies), New Delhi
Members	
GOVERNMENT ANALYST	King Institute, Madras
SHRI J. C. JAISANI	Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi
SMT DEBI MUKHERJEE (*Alternate*)	
DR E. K. JAYANARAYANAN	Mohan Meakin Ltd, Ghaziabad
SHRI RAMCHANDRA VISHVANATH KARVE	Navsari Oil Products Ltd, Navsari
SHRI ARVIND HANUMANT VIDWANS (*Alternate*)	
DR S. K. KESHYAP	Indian Council of Medical Research, New Delhi
DR D. N. ROY (*Alternate*)	
DR G. LAKSHMINARAYANA	Regional Research Laboratory (CSIR), Hyderabad
DR M. J. MULKY	Hindustan Lever Limited, Bombay
DR V. R. SHIRHATTI (*Alternate*)	
SHRI B. HANUMANTHA RAU	Bush Boake Allen (India) Ltd, Madras
DR T. S. SANTHANAKRISHNAN (*Alternate*)	
SCIENTIST	Central Food Technological Research Institute (CSIR), Mysore
DR D. G. SHAH	Kaira District Co-operative Milk Producers' Union Ltd, Anand
DR BRIJESH NARAYAN (*Alternate*)	
SHRI S. C. VASUDEVAN	Corn Products Company (India) Pvt Ltd, Bombay
SHRI S. R. PALEKAR (*Alternate*)	